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Fate by Infection: Quantifying Host Behavioral Changes in Response to Four Variables of Trematode Infection

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FATE BY INFECTION: QUANTIFYING HOST BEHAVIORAL CHANGES IN RESPONSE TO FOUR VARIABLES OF TREMATODE INFECTION

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Abstract

Although the importance of host-parasite interactions is increasingly recognized, few studies examine factors regulating how infection alters host behavior and the consequences of parasite-induced behavioral changes for predation risk. The present study assessed how infection by trematodes (parasitic flatworms) altered larval amphibian activity and whether these effects depended on trematode species, infection intensity, time post-infection, and/or host size. To examine the implications of these results for tadpole susceptibility to predation, we evaluated how infection by the most virulent trematode altered tadpole escape distance from a simulated predator. Of three trematodes (*Echinostoma trivolvis*, *Ribeiroia ondatrae* and *Alaria*), only *R. ondatrae* significantly reduced activity of Chorus frog tadpoles (*Pseudacris regilla*) one day after exposure to 40 cercariae (larval trematodes). Increased *R. ondatrae* exposure correlated with decreased host activity, especially in smaller hosts. Whether hosts were exposed to acute or chronic parasite infections influenced recovery time to normal activity. Hosts exposed to parasites in a one-time pulse returned to normal activity within five days post-infection, whereas those exposed to smaller doses of parasites daily maintained reduced activity levels for five days. Finally, infection by *R. ondatrae* reduced host escape distance from a simulated predator in a dose-dependent manner, suggesting that trematode infection could increase predation susceptibility. These results show that significant components of host behavior, including activity levels and predator avoidance, are affected by parasite infection in a manner dependent on dose, parasite identity and host traits. This underscores the significant role of parasite infection in changing host behavior and consequently species interactions.

Introduction

A parasite can directly alter the behavior of the individual (host) infected with the parasite. Some of these changes are byproducts of the infection process (trauma) and others are adaptive (increase the parasite's ability to infect a host, for example by depleting the host of resources and/or producing toxins within hosts) **(**Roberts & Janovy 2009). Parasite presence can also alter host behavior prior to infection (for example via tadpoles attempting to avoid parasites) (Taylor et al. 2004). Chemical mechanisms can cause intermediate hosts (harboring a parasite for a transition period) to become more susceptible to predation by definitive hosts (in which a parasite reaches maturity and reproduces sexually) by changing host behavior patterns. For example, the trematode *Dicrocoelium dendriticum* causes intermediate ant hosts to climb to the top of blades of grass where they are more likely to be eaten by sheep, the parasite's definitive host (Moore 2002). The latter behavioral change is the result of the parasite encysting within the ant brain (Moore 2002). Alongside systems where parasite infection changes host behavior to increase parasite transmission, as in the latter case of *D. dendriticum*, there are other, more subtle behavioral changes that can take place after exposure or infection. Examples are altered substrate preferences or hyperactivity of the host, both of which increase encounters between infected animals and predators (Moore 2002). Trauma to the host, chemically or mechanically, also is common in parasite infections. Trauma can cause a decrease in host activity, whereas initial contact with parasites can cause an increase in host activity (Taylor et al. 2004, Roberts & Janovy 2009, Daly & Johnson 2011). On the other hand, some parasites are relatively benign and cause little apparent pathology in hosts (Bull 1994). It is important to understand the dynamic effects of parasites on host behavior because host behavior can influence parasite transmission rates and parasite and host fitness (fecundity and survival) (Moore 2002).

Although many studies have demonstrated that parasites can alter host behavior (Brunner et al. 2005, Poulin 2010), relatively little is known about the factors determining the magnitude of behavioral alterations. For example, factors associated with both the parasite (e.g., species identity, virulence or degree of pathology, dosage) and the host (e.g., developmental stage, body size, exposure timing) will likely influence whether behavioral changes are observed as well as the magnitude of such changes. Despite wide variation in such factors within natural systems, most experimental studies control for these variations and thus exclude their potential importance in determining how parasites alter host behavior. Many experiments use a one-time acute exposure to parasites to test the behavioral response of hosts, while in nature hosts typically experience chronic exposure to parasites over longer durations. Similarly, few studies address the relative effects of host traits (e.g., size or developmental stage) on the degree of behavioral alteration by parasites, despite clear evidence that the timing of host exposure affects other responses such as pathology (Kelly et al. 2009, Johnson et al. 2011). Furthermore, few studies have quantified the effect of parasite-induced behavioral changes on predation susceptibility (Parris et al. 2006). Examining the role of these factors contributes to our understanding of the impact disease has on populations and communities, and helps explain the ecological consequences of parasites within ecosystems.

Background

Aquatic amphibian larvae and trematodes (parasitic flatworms) present a useful study system in which to evaluate the factors influencing parasite-induced changes in host behavior. Trematodes have complex life cycles utilizing multiple host species (Fried & Graczyk 1997). The trematodes studied here first infect a snail, wherein they reproduce asexually, producing free-swimming larval stages that leave the snail hosts and actively infect amphibians. The

trematodes complete their life cycle when the infected amphibian hosts are eaten by a bird or mammal, wherein they develop into adult worms and reproduce sexually, passing trematode eggs into water bodies with the feces of infected hosts (Fried & Graczyk 1997). Experimentally altering exposure intensity (number of parasites per host) and the timing of infection with multiple trematode species is easily accomplished because of the tractability of isolating and quantifying infectious free-living life stages.

Trematodes vary in how much pathology they cause. The trematode *Ribeiroia ondatrae* causes limb malformation and mortality in amphibians (Johnson & Hoverman 2012). *R. ondatrae* parasites infect host tadpoles by burrowing into the limb buds, causing hemorrhaging (Johnson & Hoverman 2012). Another trematode, *Echinostoma trivolvis*, ascends the cloaca (posterior opening) of a tadpole and encysts in the kidneys. *E. trivolvis* causes full-body edema (swelling) in heavy infections, but hosts are relatively asymptomatic in light to moderate infections (Johnson & Hoverman 2012). Like *E. trivolvis*, a third less commonly studied trematode in the genus *Alaria* causes no pathology in light to moderate infections. Because *Alaria* and *E. trivolvis* cause less pathology than *R. ondatrae*, the effects of a virulent parasite can be compared to two more benign parasite.

Using the trematode-amphibian system, recent studies have examined both the importance of host behavior on mediating parasite infection success and the trait-mediated effects of parasitism (the effect parasite presence has on altering host traits) (Rohr et al. 2009a, Koprivnikar 2012). Koprivnikar et al. (2006) and Daly and Johnson (2011) have found that host behavior has a strong effect on infection success. Goodman and Johnson (2011a) also found that infection and malformations strongly alter host behavior and survival (of frogs, not tadpoles). Other studies show that exposure to predators (e.g., fish) reduces tadpole activity and increases

vulnerability to trematode infection (Parris et al. 2006, Szuroczki & Richardson 2012). However, few of these studies address how infection itself alters behavior of larval amphibians and what consequences that has for predation risk.

While previous studies offer important insight into host behavior before and after infection, they did not quantify the mechanisms of infection or extend to predation susceptibility. Johnson et al. (2006) explored the relationship between parasitism and predation and observed that *Polycaryum* laeve increases the conspicuousness of Daphnia thereby increasing Daphnia predation risk. However, it remains unknown whether this relationship extends to other parasite-host systems, including the trematode-amphibian system. Additionally, few studies have examined the effects of *R. ondatrae, E. trivolvis*, and *Alaria* side-by-side.

In the current study we first tested what, if any, behavioral changes resulted from infection by three trematode species and then evaluated the role of multiple host and parasite variables (i.e., infection intensity and the timing of infection and exposure dynamics) in determining the outcome of parasite-induced behavior modifications.

Overview of experiments: We conducted four separate lab experiments examining tadpole host response to parasite infection. We sought to determine the degree of behavior modification in hosts induced by trematodes based on trematode species, exposure intensity, acute versus chronic exposure, and host size. We also examined the implications of these behavioral changes in the context of a simulated predator.

We hypothesized that after infection, hosts would exhibit little to no detectable behavioral change in response to *E. trivolvis* or *Alaria* and a significant reduction in activity level in response to the most virulent trematode, *R. ondatrae*. We expected any decreases in activity to occur for several days after infection and activity to then return to normal levels. Furthermore,

we expected host activity to return to normal after infection by *R. ondatrae* more rapidly following acute exposures than chronic exposures of the same total dosage. We predicted that larger hosts would be more resistant to behavioral changes while smaller hosts would exhibit significant reduction in activity level (Johnson et al. 2011). Finally, we expected that hosts infected by increasing dosages of *R. ondatrae* would have an incrementally shorter escape distance from a simulated predator.

Methods

Animal collection and husbandry: Larvae (tadpoles) of the Pacific chorus frog, *Pseudacris regilla*, were used as the amphibian host for all experiments, and three different species of trematode parasites: *Ribeiroia ondatrae*, *Echinostoma trivolvis,* and *Alaria*. This established study system was used because the trematode species vary in virulence and the chorus frog is widespread and often exposed to a diversity of trematodes in nature (Johnson & Hoverman 2012). All collections and experiments were performed in northern California at the Hopland Research and Extension Center, where these species are common. *P. regilla* egg masses were collected from local wetlands and tadpoles were raised in the laboratory to ensure that none were infected in nature prior to the start of the experiment. Tadpoles were fed flake fish food and dried Spirulina algae on a regular basis and did not receive any food 24 hours prior to each experiment to standardize hunger levels. *R. ondatrae, E. trivolvis,* and *Alaria* parasites were obtained from infected snails collected in the field. Each snail was placed in a water-filled 50-mL vial, and the cercariae that emerged from the snails after 4-12 hours were removed for use. Parasites were identified under a microscope using morphological characteristics (Fried & Graczyk 1997).

Experiment I: Effects of parasite identity on host activity. In the first experiment, the effects of infection by different trematodes on host behavior were tested using four treatments: 1)

control (uninfected hosts); 2) exposure to 40 *R. ondatrae* cercariae; 3) exposure to 40 *E. trivolvis* cercariae; and 4) exposure to 40 *Alaria* cercariae. Tadpoles were placed individually into containers filled with 750 mL of aged mesocosm water. Exact counts of cercariae were placed in the water with each host and left for 15-24 hours to allow time for them to infect hosts and encyst. Host behavior was monitored 10 minutes before infection and one-day (15-24 hours) post infection. Because *R. ondatrae* emerges from snail hosts later in the day than *E. trivolvis* and *Alaria*, *R. ondatrae* exposures were performed at 23:00 hours, *E. trivolvis* and *Alaria* exposures were performed at 14:00 hours on the same day, and one day post infection observations were performed the following day at 14:00 hours) (Johnson & Hoverman 2012). Behavioral recording took place in the lab where each host was observed for three seconds and whether it moved was recorded (movement was the measure for activity). All replicates were observed one after another, and then the order of observations was reversed for the following observation period. All tadpoles were observed for three seconds. The process was repeated a total of 30 times for the entire set of replicates, and the average number of observations in which movement was detected (of 30 total) was used as the primary response variable.

Experiment II: Effects of parasite dosage on host activity and recovery time. In the second experiment, the effects of parasite dosage and time after infection on host behavior were tested. The differences in activity level between hosts infected during a one-time acute exposure versus a daily, chronic exposure of the same cumulative dosage were also examined. The experiment included seven treatments consisting of 10 individual tadpoles each. Treatments one through six involved single acute exposures to 0, 5, 10, 20, 30, and 40 cercariae, respectively. Treatment seven involved exposure to 10 *R. ondatrae* cercariae every day for four days (chronic exposure). Host behavior was monitored in all treatments for four days prior to exposure and for the six

days following. Observations were performed daily at 8:00 hours. Tadpoles in treatments two through six were exposed to cercariae one time on day four of the experiment. For treatment seven, hosts were infected with 10 cercariae every day for four days, starting on day four of the experiment. The methods for performing infections and monitoring behavior in experiment II were identical to I.

Experiment III: Effects of host size on parasite-induced behavioral changes. In experiment III, how tadpole size and developmental stage influence parasite-induced behavioral alteration was tested. This experiment included four treatments of 10 individual tadpoles each. The treatments consisted of tadpoles falling into four size classes: 1) Gosner (standard table to define developmental stages of frogs) stage ~26; 2) stage ~27; 3) stage ~30 tadpoles; and 4) stage ~34 tadpoles (Gosner 1960). The snout-vent length (SVL) of all tadpoles was also measured and included as a covariate. In all treatments, each host was exposed individually to 20 *R. ondatrae* cercariae within 750 ml of aged mesocosm water. The methods for performing infections and monitoring behavior in experiment III were identical to I. Behavior of each tadpole was monitored 10 minutes before exposure and one day post-exposure. All observations were performed at 14:00 hours.

Experiment IV: Effects of parasite exposure on host responses to a simulated predator. In experiment IV, how infection intensity influenced the escape distance of a tadpole to a simulated predator was tested. The experiment included four treatments, each consisting of 10 individual tadpoles. The treatments were Gosner stage 27 tadpoles exposed to 0 (control), 5, 10, or 20 *R. ondatrae* cercariae. Tadpoles were exposed to the designated number of parasites in 750 mL of water for 12 hours to allow as many parasites as possible to encyst in each tadpole. Escape distance of the different treatments after parasite infection were compared after subsequently

confronting each tadpole with a simulated predator ~12 hours after infection. This was done by placing each tadpole facing forward in an experimental runway (see Fig. 1 for dimensions), waiting 10 seconds for the tadpole to cease movement, and then recording the individual's "start" position using a ruler. Then, a popsicle stick was carefully inserted close to (but not touching) the tadpole to act as a simulated threat (Goodman & Johnson 2011b). The "end" position of the tadpole was recorded at the point where it first stopped moving after fleeing from the simulated predator. There were three trials performed with each tadpole, with a 30-minute rest period between trials.

Analysis: Each dataset was analyzed with a linear mixed-effects model using the nlme package in R (R Development Core Team 2008, Zuur et al. 2009). In experiment I, activity level was specified as the response (the mean of 30 observations), trematode species, experimental period (before and after exposure), and their interaction as fixed effects, and individual host as a random effect. In experiment II, parasite dosage, experimental period, and their interaction were specified as fixed effects, and individual host as a random effect. In experiment III, host size (SVL), experimental period, and their interaction were specified as fixed effects, and individual host as a random effect. In experiment IV, parasite dosage was specified as a fixed effect and individual host as a random effect. Because there were three trials for each tadpole in experiment IV, a random effect of trial nested within tadpole was also included.

Results

Experiment I: Effects of parasite identity on host activity. Of the three common trematodes tested, only *R. ondatrae* induced significant behavioral changes in *P. regilla* hosts at a dosage of 40 parasites (Fig. 2). *E. trivolvis* and *Alaria* induced no significant changes in host behavior, with hosts being active approximately 25% of the time pre-exposure, and 35% of the time post-

exposure. In contrast, hosts exposed to *R. ondatrae* were seven times less active one day after exposure relative to pre-infection activity levels. There was a significant interaction between the *R. ondatrae* treatment and experimental period, indicating that changes in activity manifested only after parasite exposure had occurred (i.e., there were no differences among tadpoles in different treatments prior to parasites being administered) (Fig. 2).

Experiment II: Effects of parasite dosage on host activity and recovery time. The intensity of *R*. *ondatrae* exposure (from 0 to 40 cercariae) influenced the degree of behavioral reduction in a dose-dependent manner, with higher infection levels causing progressively greater decreases in activity (Fig. 3). One day after infection, hosts exposed to 40 *R*. *ondatrae* cercariae were over two-and-a-half times less active than those exposed to 10 cercariae and over three times less active than those exposed to only five cercariae (Fig. 3). This effect was time-dependent, such that behavior returned to pre-infection levels within five days post exposure in all acute exposure treatments. Hosts exposed to low doses of parasites recovered more quickly than those exposed to higher doses. There was an interaction between dosage and day. When hosts were subject to chronic daily exposure of 10 *R*. *ondatrae* cercariae for four days, their activity remained reduced for the duration of the exposures (5 days).

Experiment III: Effects of host size on parasite-induced behavioral changes. Experimental period and host size (SVL) interacted significantly to determine parasite-induced changes in host behavior with smaller hosts experiencing stronger reductions in behavior following infection. Hosts in the two smallest size classes (stage 26-27) showed a 48% and 53% decrease, respectively, in activity relative to pre-infection levels. Hosts in the two largest size classes (~stage 29-37) showed a 12% and 24% increase in activity, respectively (Fig. 4). There were no significant interactions. Infection intensity was not significantly different between treatments.

Dissections of all tadpoles after the experiment indicated that, on average, 17.4 parasites encysted in tadpoles of the smallest size class, 18.7 and 17.7 parasites in the middle two size classes, and 16.3 in the largest size class.

Experiment IV: Effects of parasite exposure on host responses to a simulated predator. Tadpole escape distance in response to a simulated predator decreased monotonically with increasing parasite load. Tadpoles exposed to 20 cercariae travelled a distance five times shorter than unexposed control tadpoles (Fig. 5). There were no significant interactions.

Discussion

The present finding that hosts exposed to *R. ondatrae* were seven times less active following exposure relative to pre-infection activity levels is likely a result of the pathology caused by the large size of *R. ondatrae* and its use of direct penetration entry into the host, in which it uses proteolytic enzymes to burrow into the limb buds and causes hemorrhaging (Johnson et al. 1999, Johnson et al. 2004). The host expends energy to recover from associated tissue damage and is thus less active. The fact that neither of the other two trematodes induced detectable changes in host behavior at the administered dosage may stem from both mode of entry and relative body size. *E. trivolvis* enters its amphibian host via the cloaca and does not cause severe injury at the entry site. *Alaria* penetrates the body much like *R. ondatrae*, but its small size may limit its pathology and subsequent effect on behavior (Rohr et al. 2009b, Johnson et al. 2011, Johnson & Hoverman 2012). A caveat in the present study is that total dosages were not varied for any parasites other than *R. ondatrae*, thus leaving the question unanswered of whether a higher dosage of *E. trivolvis* or *Alaria* would affect host behavior. It has been shown that at high doses, *E. trivolvis* causes edema in hosts, which reduces activity levels. Infection intensity varies greatly in nature. The present study involved intensities ranging from 0-40

parasites, which is a reasonable level to find in nature; however infections close to 1,000 parasites of each of the three species used can also be found in nature (Johnson & McKenzie 2008, Daly & Johnson 2011).

Our findings indicated that both parasite dosage (number of cercariae to which a host was exposed) and the size of the host at the time of exposure influenced the magnitude of *Ribeiroia* effects on behavior. Both dose-dependent reduction in behavior and increase in recovery time are consistent with the argument that mechanical damage by the parasite is responsible for the reduction of tadpole activity levels after infection and with the previous finding that pathology increased with infection intensity (Brunner et al. 2005).

In natural systems hosts are typically exposed to parasites chronically. Hosts exposed to a daily exposure of *R. ondatrae* for four days showed reduction in activity that persisted for all four days of infection plus the remainder of the experiment. It would be valuable to perform a similar experiment where behavior was monitored at least five days after the final chronic dose was administered to allow comparison of the present data to the recovery period of hosts exposed to the whole dosage of parasites at once.

Alongside variation in parasite characteristics, the present study varied traits of the host, including body size and developmental stage and found smaller hosts to be associated with greater reductions in activity level in response to infection by *R. ondatrae*. When it comes to the effect of host size on susceptibility to parasite infection, it is important to take into consideration what mechanism is driving the response. While larger hosts experienced less dramatic behavioral changes in response to parasite exposure, it remains unclear whether this was a function of larger hosts preventing parasites from encysting or the result of faster host recovery post infection. In the present study, parasites had similar success rates in all host sizes. This lack of variation,

which probably stems from the small volume of water in which tadpoles were exposed to tadpoles, suggests that differences in infection success are unlikely to be the mechanism responsible for observed changes in behavior. Instead, smaller and less developed hosts likely suffer a greater magnitude of parasite-induced tissue damage relative to larger and more mature individuals. Correspondingly, Johnson et al. (2011) found that amphibian pathology driven by trematode infection depends on amphibian developmental stage. There is a critical window during pre-limb and early limb developmental stages, during which a host is most likely to experience infection and pathology. Host developmental stage not only influences changes in resistance (infection upon exposure) but also changes in tolerance (level of pathology following infection) (Johnson et al. 2011).

The present results have implications beyond the laboratory, because the behavioral responses may influence hosts in a natural environment, thus impacting the food web dynamics as a whole. The present finding that, with increasing parasite load, tadpole escape distance from a simulated predator decreased, indicates that *R. ondatrae* infections may have direct implications for predator-prey relationships. Depending on the predator, these impacts may make tadpoles more or less susceptible to predation—less active tadpoles may be less likely to be detected by a predator (e.g. dragonfly larvae, fish) but, when detected, we find they escape a shorter distance. This idea can be explored going forward by designing an experiment where tadpoles are exposed to a natural predator, to examine how behavioral changes associated with parasite infection influence predation susceptibility (Parris et al. 2006, Szuroczki & Richardson 2012).

Our results are consistent with other studies of trematode virulence. Similar studies have shown the relatively strong virulence of *R. ondatrae* and confirmed that parasite dosage is

positively correlated with suppression of host activity (Rohr et al. 2009b). Other studies concur that stage of development is important in parasite infection and that there is a critical window of disease risk. In our study comparing different size hosts, all hosts were at developmental stages (Gosner stages 26-37) where infection by *R. ondatrae* is highly successful (Johnson et al. 2011). Our study shows that, while all hosts existed in this critical range, smaller hosts were still more likely to exhibit reduced activity levels. Our finding that size did not change parasite success rate is likely due to small volume of water used in exposure trials.

Although the existing paradigm suggests parasites evolve into less virulent forms because, if the host dies, parasite survival decreases; however theories suggest parasites evolve an "optimal virulence" to maximize survival rate (Lenski & May 1994, Frank 1996). Optimal virulence depends on factors such as host and parasite genetic diversity, specific host-parasite interactions, pathogenicity, parasite life span and transmission dynamics (Lenski & May 1994). In the case of trematodes, we discovered quantifiable differences among trematode species. However, it remains unknown whether behavioral changes due to *R. ondatrae* infection observed here are adaptive for the parasite or "side effects" of infection. Our study showed that a decrease in host activity resulting from a *R. ondatrae* infection reduces tadpole escape distance from a simulated predator, possibly making tadpoles more vulnerable to predation. This would not necessarily benefit the parasite, however, because the *R. ondatrae* life cycle requires a predator that is a suitable host to prey on a tadpole or frog (i.e. a bird or mammal). Aquatic predators of tadpoles such as dragonfly larvae and fish do not advance the *R. ondatrae* life cycle. On the other hand, predators such as dragonfly larvae tend to attack tadpoles that exhibit movement, because dragonflies are visual predators. In this case, a tadpole with subdued activity may be less susceptible to predation. Furthermore, this interaction may depend on the type of predator

(ambush vs. tactile).

It would be interesting to compare success rates for *R. ondatrae*, *E. trivolvis*, and *Alaria* in nature, because this should provide insight into whether the relatively strong virulence of *R. ondatrae* is effective in perpetrating the parasite life cycle or if it is detrimental. Future studies should make lab experiments on host-parasite interactions more realistic by using a predator-prey system rather than a simulated predator. It would also be valuable to extend our investigation to other systems.

Our results reinforce our understanding of the factors that regulate how infection alters host behavior and extend our understanding of the consequences parasite-induced behavioral changes have on predation risk. We identified the importance of parasite dose and the identity of the parasite involved as well as traits of the host in determining host behavioral changes. Further, studies to examine how parasite-induced changes in host behavior alter other components of population, community, and ecosystem ecology are warranted within our study system. For instance, behavioral alterations are likely to alter host growth and reproduction and the transmission of *Ribeiroia* to definitive hosts. Furthermore, trait-mediated effects of trematode parasites on primary production are likely, but unstudied in this system. Taken together, our results further the integration of disease into the study of community ecology and show that parasite infection can play a significant role in changing host behavior and consequently species interactions within freshwater food webs.

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Literature Cited

Brunner J. L., Richards K., & Collins J. P. (2005). Dose and host characteristics influence virulence of ranavirus infections. *Oecologia* 144:399–406.

Bull J. J. (1994). Perspective virulence. *Evolution* 48:1423-1437.

Daly E. W., & Johnson P. T. J. (2011). Beyond immunity: quantifying the effects of host anti parasite behavior on parasite transmission. *Oecologia* 165:1043-1050.

Frank S. A. (1996). Models of parasite virulence. *Quarterly Review of Biology* pp. 37-78.

Fried B., & Graczyk T. K. (1997). *Advances in Trematode Biology*. Boca Raton, FL: CRC Press.

Goodman B. A., & Johnson P. T. J. (2011a). Disease and the extended phenotype: parasites control host performance and survival through induced changes in body plan. *PLoS ONE* 6:e20193.

Goodman B. A., & Johnson P. T. J. (2011b). Ecomorphology and disease: understanding the cryptic effects of parasitism on host habitat use, thermoregulation, and predator avoidance. *Ecology* 92:542-548.

Gosner K.L. (1960). A simple table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16:183–190.

Johnson P. T. J., Lunde K. B., Ritchie E. G., & Launer A. E. (1999). The effect of trematode infection on amphibian limb development and survivorship. *Science* 284:802–804.

Johnson P. T. J., Sutherland D. R., Kinsella J. M., & Lunde K. B. (2004). Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. *Advances in Parasitology* 57:191-253.

Johnson P. T. J., Stanton D. E., Preu E. R., Forshay K. J., & Carpenter S. R. (2006). Dining on

disease: how interactions between infection and environment affect predation risk. *Ecology* 87:1973-1980.

Johnson P. T. J., & McKenzie V. J. (2008). Effects of environmental change on helminth infections in amphibians: exploring the emergence of *Ribeiroia* and *Echinostoma* infections in North America. Chapter 11 in Fried, B. and R. Toledo (Eds.).(2009). The Biology of Echinostomes, New York, NY: Springer.

Johnson P. T. J., Kellermanns E., & Bowerman J. (2011). Critical windows of disease risk: amphibian pathology driven by developmental changes in host resistance and tolerance. *Functional Ecology* 25:726-734.

Johnson P. T. J., & Hoverman J. T. (2012). Parasite diversity and coinfection determine pathogen infection success and host fitness. *Proceedings of the National Academy of Sciences* 109:9006-9011.

Kelly D.W., Thomas H., Thieltges D.W., Poulin R., & Tompkins D.M. (2009). Trematode infection causes malformations and population effects in a declining New Zealand fish. *Journal of Animal Ecology*, 79:445–452.

Koprivnikar J., Forbes M. R., & Baker R. L. (2006). On the efficacy of anti-parasite behaviour: a case study of tadpole susceptibility to cercariae of *Echinostoma trivolvis. Canadian Journal of Zoology* 84:1623-1629.

Koprivnikar J., Gibson C. H., & Redfern J. C. (2012). Infectious personalities: behavioural syndromes and disease risk in larval amphibians. *Proceedings of the Royal Society of London Series* B 279:1544-1550.

Lenski R. E., & May R. M. (1994). The evolution of virulence in parasites and pathogens: reconciliation between two competing hypotheses. *Journal of Theoretical Biology*, 169(3):253-265.

Moore J. (2002). *Parasites and the Behaviour of Animals. (Oxford Series in Ecology and Evolution)*. New York, NY: Oxford University Press.

Parris M. J., Reese E., & Storfer A. (2006). Antipredator behavior of chytridiomycosis-infected northern leopard frog (*Rana pipiens*) tadpoles. *Canadian Journal of Zoology* 84(1):58-65.

Poulin R. (2010). Parasite manipulation on host behavior: an update and frequently asked questions. *Advances in the Study of Behavior* 41:151-186.

R Development Core Team (2008). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Roberts L. S., & Janovy Jr. J. (2009). Gerald D. Schmidt & Larry S. Roberts' foundations of parasitology. (8th ed.). Dubuque, IA: McGraw-Hill.

Rohr J. R., Swan A., Raffel T. R., & Hudson P. J. (2009a). Parasites, info-disruption, and the ecology of fear. *Oecologia* 159:447–454.

Rohr J. R., Raffel T. R., & Sessions S. K. (2009b). Digenetic trematodes and their relationship to amphibian declines and deformities. In: Heatwole H, Wilkinson JW, eds. Amphibian Biology, Volume 8, Amphibian Decline: Diseases, Parasites, Maladies and Pollution. Chipping Norton, NSW, Australia: Surrey Beatty & Sons, Chapter 4, pp. 3067-3088.

Szuroczki D., & Richardson J. M. L. (2012). The behavioral response of larval amphibians (*Ranidae*) to threats from predators and parasites. *PLoS ONE* 7(11):e49592.

Taylor C. N., Oseen K. L., & Wassersug R.J. (2004). On the behavioural response of *Rana* and *Bufo* tadpoles to echinostomatoid cercariae: implications to synergistic factors influencing trematode infections in anurans. *Canadian Journal of Zoology* 82:701–706.

Zuur A. F., Ieno E. N., Walker N. J., Saveliev A. A., & Smith G. M. (2009). Mixed effects models and extensions in ecology with R. New York, NY: Springer.

Figure Legends

Figure 1. Experimental setup for experiment IV, which consisted of a 7.62 cm wide, 100 cm long gutter beside which was a meter stick to measure tadpole distance travelled.

Figure 2. Percentage of hosts active pre-infection and one day post infection following exposure to 40 cercariae of one of three different trematodes, *Alaria*, *E. trivolvis*, and *R. ondatrae*. GLMM, *R. ondatrae* $z = -4.58$, $p < 0.001$; *E. trivolvis* $z = 1.01$, $p = 0.32$; *Alaria* $z = 0.58$, $p = 0.56$; treatment:period $z = 3.64$, $p < 0.001$.

Figure 3. Percentage of hosts active pre-infection, one-day post infection, and 5 days post infection following exposure to varying intensities of *R. ondatrae* cercariae. GLMM, dosage:day $z = -3.34$ $p < 0.001$.

Figure 4. Percent change in activity level of four different size classes of *P. regilla* hosts in response to *R. ondatrae* infection. GLMM, time:size $z = -3.25$, $p = 0.002$.

Figure 5. Centimeters travelled by hosts exposed to 0, 5, 10, and 20 *R. ondatrae* cercariae in response to a simulated predator. GLMM, $z = -4.64$, $p < 0.001$.

Figure 1.

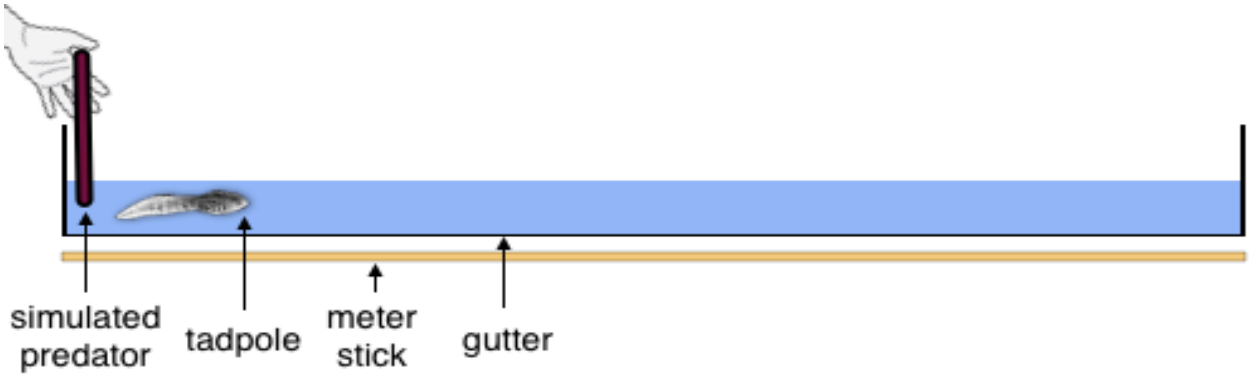


Figure 2.

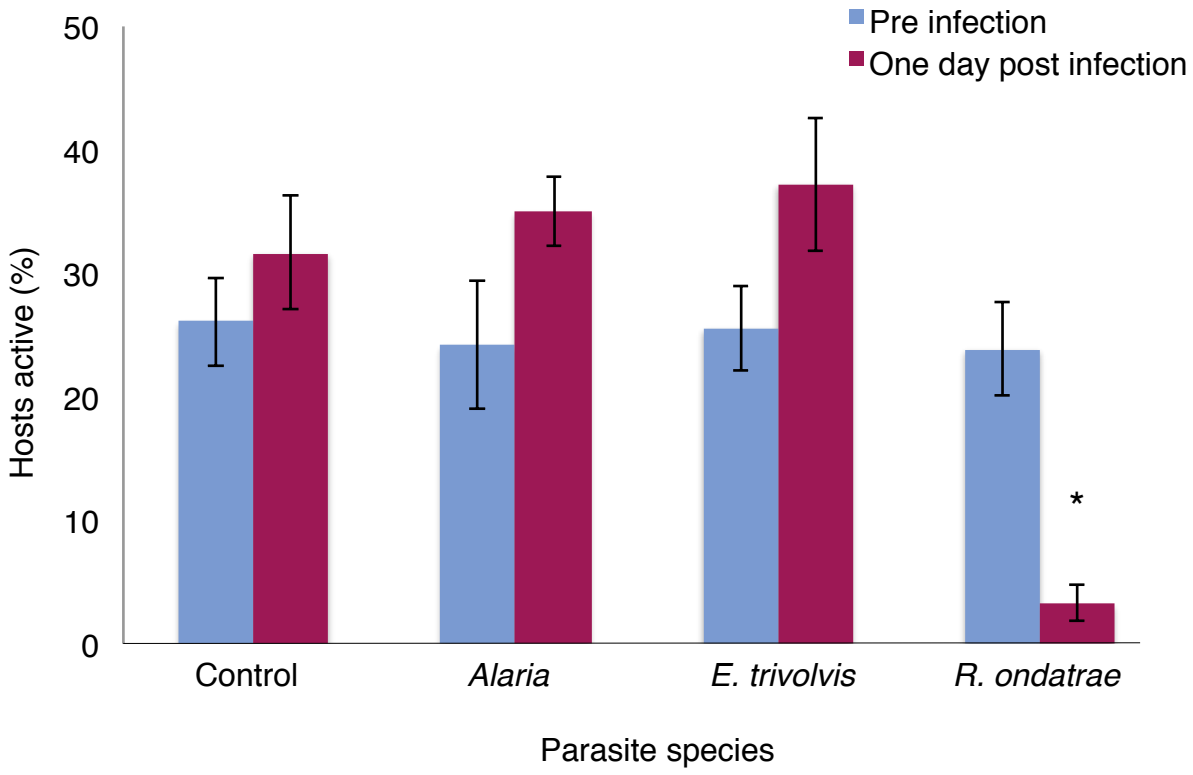

Pre infection
One day post infection
Hosts active (%)
50
40
30
20
10
0
*
Control
Alaria
E. trivolvis
R. ondatrae
Parasite species

Figure 3.

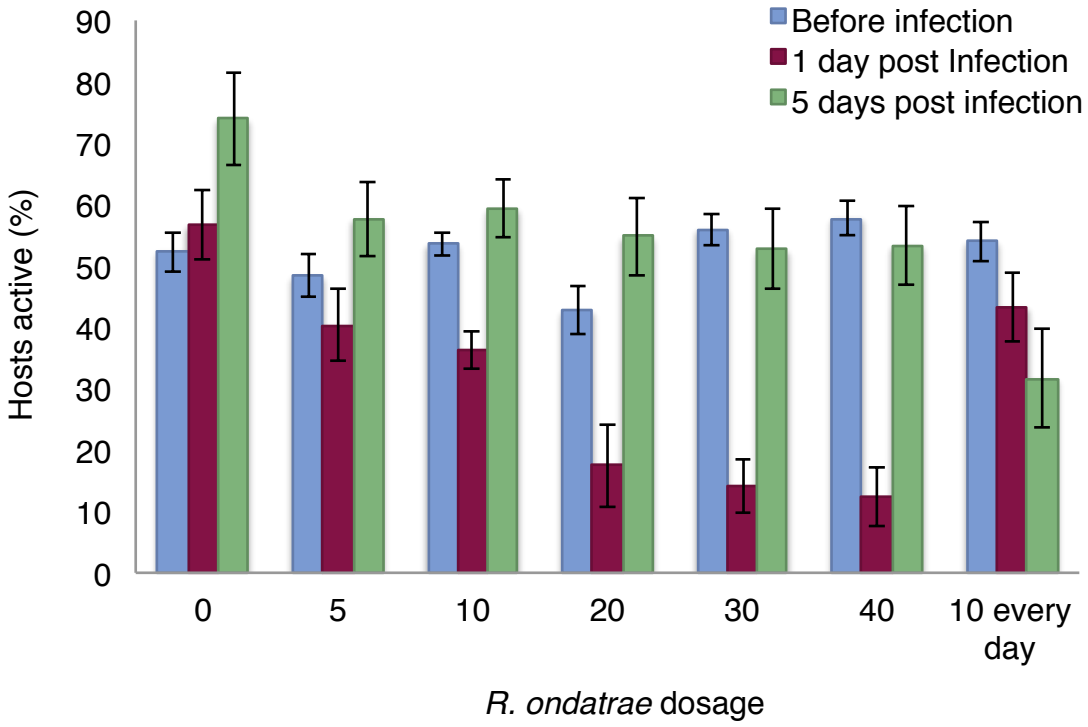

Before infection
1 day post Infection
5 days post infection
Hosts active (%)
90
80
70
60
50
40
30
20
10
0
0
5
10
20
30
40
10 every day
R. ondatrae dosage

Figure 4.

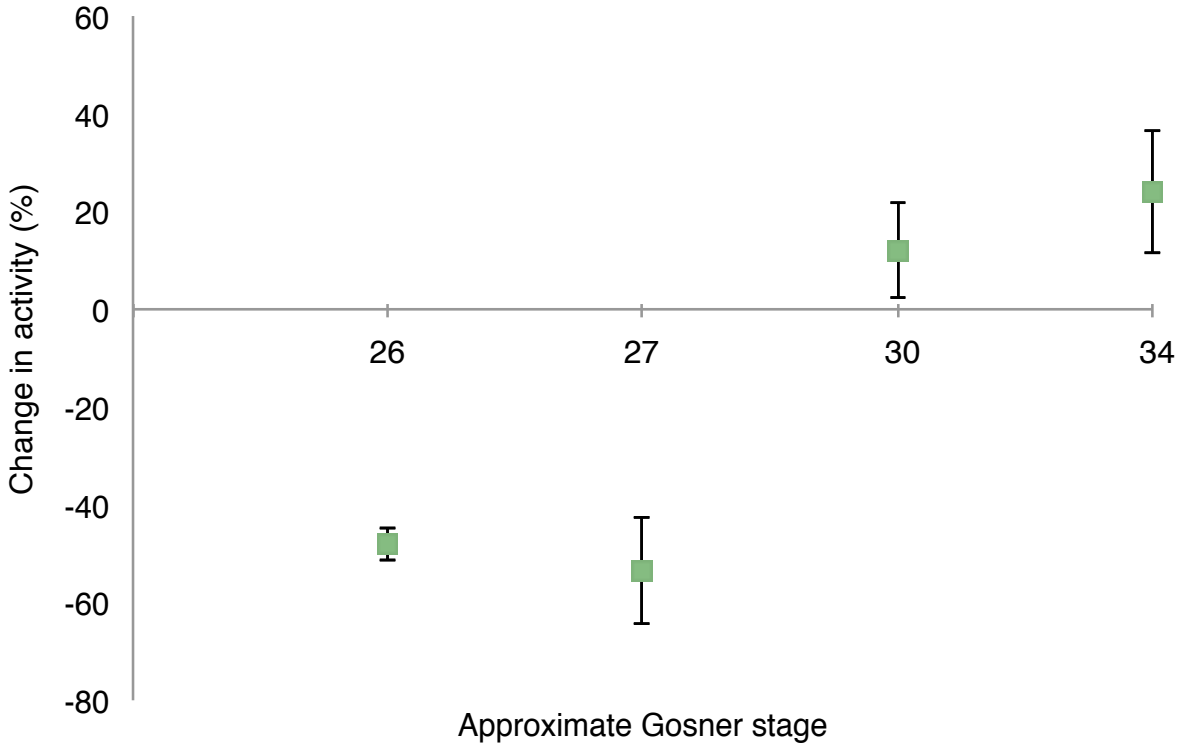
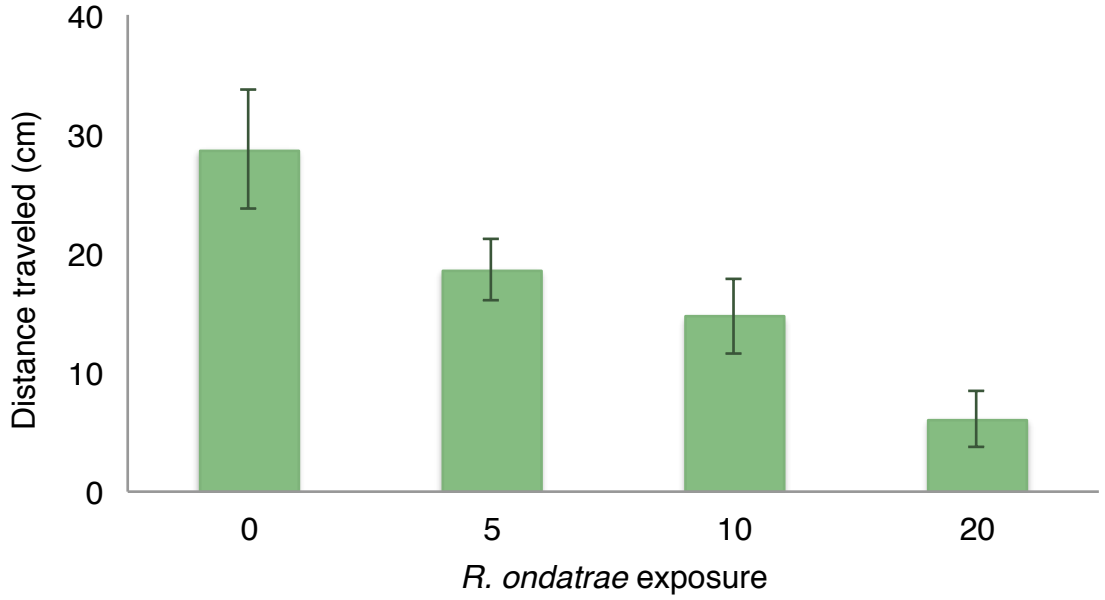

60
40
20
0
-20
-40
-60
-80
Change in activity (%)
26
27
30
34
Approximate Gosner stage

Figure 5.


40
30
20
10
0
Distance traveled (cm)
0
5
10
20
R. ondatrae exposure